

Amendments to the Claims

The listing of claims will replace all prior versions, and listings, of claims in the above-identified application.

Listing of Claims

Claims 1 - 34 **(Cancelled)**.

35. **(Original)** A method of determining protease activity of botulinum toxin serotype A or serotype E (BoNT/A/E), comprising the steps of:

- (a) treating a sample, under conditions suitable for clostridial toxin protease activity, with a BoNT/A or BoNT/E substrate comprising
 - (i) a donor fluorophore;
 - (ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and
 - (iii) a BoNT/A or BoNT/E recognition sequence comprising a cleavage site,

wherein said cleavage site intervenes between said donor fluorophore and said acceptor and wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor;

- (b) exciting said donor fluorophore; and
- (c) determining resonance energy transfer of said treated substrate relative to a control substrate,

wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A or BoNT/E protease activity.

36. **(Original)** The method of claim 35, wherein said botulinum toxin substrate is a BoNT/A substrate comprising a BoNT/A recognition sequence.

37. **(Withdrawn)** The method of claim 35, wherein said botulinum toxin substrate is a BoNT/E substrate comprising a BoNT/E recognition sequence.

38. **(Original)** The method of claim 35, wherein said sample is a crude cell lysate.

39. **(Original)** The method of claim 35, 36 or 37, wherein said sample is isolated clostridial toxin.

40. **(Original)** The method of claim 35, 36 or 37, wherein said sample is isolated clostridial toxin light chain.

41. **(Original)** The method of claim 35, wherein said sample is a formulated clostridial toxin product.

42. **(Currently amended)** The method of claim 35, wherein said sample is formulated BoNT/A product containing human serum albumin [[BOTOX®]].

43. **(Original)** The method of claim 35, step (c) comprising detecting donor fluorescence intensity of said treated substrate,

wherein increased donor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

44. **(Original)** The method of claim 35, step (c) comprising detecting acceptor fluorescence intensity of said treated substrate,

wherein decreased acceptor fluorescence intensity of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

45. **(Original)** The method of claim 35, step (c) comprising detecting an acceptor emission maximum and a donor fluorophore emission maximum,

wherein a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum is indicative of clostridial toxin protease activity.

46. **(Original)** The method of claim 35, step (c) comprising detecting the ratio of fluorescence amplitudes near an acceptor emission maximum to the fluorescence amplitudes near a donor fluorophore emission maximum,

wherein a decreased ratio of said treated sample as compared to said control sample is indicative of clostridial toxin protease activity.

47. **(Original)** The method of claim 35, step (c) comprising detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increased donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate is indicative of clostridial toxin protease activity.

48. **(Original)** The method of claim 35, further comprising repeating step (c) at one or more later time intervals.

49. **(Currently amended)** The method of claim 35, wherein at least 90% of said **[[clostridial toxin]]** **BoNT/A or BoNT/E** substrate is cleaved.

50. **(Currently amended)** The method of claim 35, wherein at most 25% of said **[[clostridial toxin]]** **BoNT/A or BoNT/E** substrate is cleaved.

51. **(Currently amended)** The method of claim 50, wherein at most 15% of said **[[clostridial toxin]]** **BoNT/A or BoNT/E** substrate is cleaved.

52. **(Currently amended)** The method of claim 51, wherein at most 5% of said **[[clostridial toxin]]** **BoNT/A or BoNT/E** substrate is cleaved.

53. **(Original)** The method of claim 35, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

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CONCLUSION

The Examiner is invited to contact the undersigned agent with any questions related to this application.

Respectfully submitted,

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